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January 25, 2005

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APPLICATION NUMBER: *60/532,681*
FILING DATE: *December 23, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/43499*

Certified by

Jon W. Dudas

Under Secretary of Commerce
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18379 U.S.PTO

PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 389269196

2138B U.S.PTO 60/532681

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Vijay K. Song-Hua	Mahant Ke	Murrieta, CA San Diego, CA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Biochip Configurations and Methods

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] Customer Number: 34284

OR

[ ] Firm or Individual Name					
Address					
Address					
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Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 5
[X] Drawing(s) *Number of Sheets* 1
[ ] Application Data Sheet. See 37 CFR 1.76
[ ] CD(s), Number _____
[ ] Other (specify) _____

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.
[ ] A check or money order is enclosed to cover the filing fees.
[X] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502191
[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Martin Fessenmaier

TELEPHONE 714-641-5100

Date 12/23/03

REGISTRATION NO. 46697 *(If appropriate)*

Docket Number: 100788.0022PRO

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	December 23, 2003
First Named Inventor	Vijay K. Mahant
Examiner Name	
Art Unit	
Attorney Docket No.	100788.0022PRO

METHOD OF PAYMENT *(check all that apply)*

[ ] Check [ ] Credit card [ ] Money Order [ ] Other [ ] None

[X] Deposit Account:

Deposit Account Number: 502191

Deposit Account Name: Rutan & Tucker

The Director is authorized to: *(check all that apply)*

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY

(Complete *if applicable*)

Name (Print/Type)	Martin Fessenmaier	Registration No. (Attorney/Agent)	46697	Telephone	714-641-5100
Signature				Date	December 23, 2003

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BIOCHIP CONFIGURATIONS AND METHODS

Field of The Invention

Analytic devices and methods, particularly as they relate to analytic devices and methods using biochips.

Background of The Invention

Thousands of clinical tests are available for routine check-ups, diagnosis of conditions and diseases, and many physicians rely heavily on the test results to establish a diagnosis and/or treatment plan. Consequently, accuracy and reliability of such methods are of paramount importance.

In numerous cases, calibration materials and controls are used as tools to assess accuracy and precision of test results, and typically provide at least some information once a test protocol has been executed. Recently, an active quality process control program for continuous control of an analytic process with real-time automated error detection/correction, and documentation of corrective actions was introduced (iQMTM on the GEM®Premier 3000). Such systems are often reliable and provide accurate error reports, however, are typically difficult to integrate into microarray-based assays. One of the major challenges in almost all microarray-based test systems is the relatively strong variance of the system due to poor spotting quality, imprecise micro-movements of motors or mechanical parts for scanning, and fluctuations in power, light sources and/or detectors (*e.g.*, PMT).

Moreover, and especially where microarray systems are employed for nucleic acid analysis, relatively expensive reagents and pre-amplification steps are often required to obtain a reliable and quantifiable result from a test sample. For example, a number of primer extension methodologies are known (see below) in the art, and are typically performed as single nucleotide primer extension. However, such methods generally require expensive detectable dye-labeled terminators such as acylo- or dideoxy nucleotides, and often need to be performed with pre-amplified material.

Therefore, although there are numerous diagnostic devices and methods known in the art, there is still a need to provide improved biochip configurations and methods for analytic and diagnostic devices.

Detailed Description

The inventors discovered that numerous assays can be performed on a biochip with increased accuracy and simplicity using a biochip that includes a capture probe having an intrinsic reference marker, and or using a capture probe that serves as a substrate in a non-restricting enzymatic assay. While it is generally contemplated that the configurations and methods according to the inventive subject matter may be implemented with any biochip, particularly preferred biochips are those described in our co-pending patent applications with the serial numbers 10/346879 (filed 1/17/03) and 10/433766 (filed 10/10/03), both of which are incorporated by reference herein.

Tagged Capture Probes

In one particularly preferred aspect of the inventive subject matter, a capture probe is tagged with a fluorophore, chemiluminophore, or otherwise detectable label, and the capture probe is immobilized in a predetermined position on the biochip. Thus, especially contemplated capture probes include antibodies, antigens, or nucleic acids (*e.g.*, DNA or RNA oligos). Typically, the capture is immobilized on a solid-phase (*e.g.*, glass, plastic or a membrane) or on/in a matrix.

It should be especially appreciated that the tagged capture probes can be used as internal quality control to check the quality and dimensions of the spots. Moreover, the tagged probes can also be exploited to design assays based on ratio determination. For example, in a sandwich immunoassay, the capture antibody can be tagged with the Cy3 dye while the reporter antibody is tagged with the Cy5 dye.

Therefore, an exemplary method of quantitative and/or qualitative analysis may include a step of tagging a capture probe with a detectable label. In another step, the tagged capture probe is immobilized on a biochip in a predetermined position (*e.g.*, on a solid-phase). In yet another step, unlabeled or labeled sample and/or a reporter probe is added to the tagged capture probe,

and hybridization or incubation of the sample is performed with the tagged capture probe and/or in the presence of the reporter probe. In still a further step, the labels of the tagged probes (and the reporter probes) are detected and quantified using suitable technologies (*e.g.*, chemically, spectroscopically, or electrochemically).

In further especially preferred aspects, the label (*i.e.*, the tag) is at least one of a fluorophore, a chemiluminophore, and an electroactive group, while the tagged capture probe is preferably an antibody, an antigen, or an oligonucleotide. Where reporter probes are employed, the reporter probe is advantageously an antibody, an antigen, or an oligonucleotide. Of course, it should be recognized that depending on the chemical nature of the tag and/or the capture probe, the tag can be covalently or non-covalently coupled to the probe. Furthermore, it should be appreciated that the tag on the tagged capture probe can be used during spotting for positional control, and/or control of the amount deposited onto the biochip. during spotting.

For additional control (*e.g.*, bleaching, quenching, identification, etc.) an additional tag can be immobilized without capture probe onto the biochip. Moreover, the tag on the capture probe may also be employed for quality control and ratio determination in an assay. Most preferably, spot morphology of the spot can be determined (*e.g.*, dimension of the spot).

Solid-Phase Primer Extension

The present inventive subject matter also relates to methods and reagents for determining the presence of a specific nucleotide in a specific position based on a microarray polynucleotide primer extension. In especially preferred aspects of such methods and reagents, the microarray polynucleotide primer extension does not require the use of dye-labeled chain terminators (*e.g.*, acylo-, or dideoxy nucleotides.

Primer extension as practiced herein is a result of polymerization of the complimentary deoxynucleotide and a detectable deoxynucleotide, and is not limited to the complimentary first base but proceeds up to the length of the available target and then terminates automatically. The primer extension based labeling without the use of terminators is an efficient way to label nucleic acids using microarray or solid-phase based technologies. Target specific hybridization is performed to achieve assay specificity.

Exemplary Protocol for on-chip non-limiting Primer Extension:

(1) Prepare a DNA chip with specific capture oligonucleotides as shown in Figure 1.

(2) Hybridize the immobilized capture oligonucleotides with 60 ul of hybridization solution (2.5 X SSC, 0.2% Tween 20, 12.5 mM HEPES, pH 7.5, 30 % Formamide) containing CFTR template DNA and CB control DNA at 37 degree Celsius for 45 minutes.

(3) After removing the hybridization solution, wash the chip with 100 ul of 1X SSC, 0.2% SDS one times and 0.5 X SSC solution twice at room temperature.

(4) Apply primer extension solution 50 ul onto the chip and incubate at 37 degree for 30 min. (Primer extension solution: 0.5 uM of each dATP, dTTP and dGTP, 0.5 uM of Cy5 labeled dCTP, 1 unit of Therminator DNA polymerase and 1x reaction buffer).

(5) Remove the reaction mixture, and wash the chip three times with 100 ul of 10 mM Tris HCl, 0.2 % Tween 20.

(6) Excite the chip with lasers for Cy3 and Cy5 dyes and detect emission fluorescence with a photomultiplier tube (PMT). The result using On-Chip Labeling using Primer Extension with Cy5 dCTPis shown in Figure 2.

Therefore, a method for determining a specific nucleotide at one or more location in a target nucleic acid may include one step in which a single stranded target nucleic acid is provided. In another step, the target nucleic acid is hybridized with a primer complimentary to the target nucleic acid, wherein the 3'-end of the primer binds to nucleotides flanking the specific nucleotides at sites in the target nucleic acid. In still another step, the hybridized nucleic acid is exposed to a polymerase in a mixture containing at least one deoxynucleotide and one or more labeled deoxynucleotide.

In preferred aspects, the target nucleic acid is obtained from a human, animal, virus, bacteria, plant, or fungus (most preferably from a living specimen), and the above described method is used for determination of specific nucleotide in predetermined position, species

identification, identification of diseases, predisposition to genetic diseases, fingerprinting, and/or pharmacogenomics.

Particularly preferred method will typically not require amplification of the target nucleic acid, but amplification is not excluded (*e.g.*, via polymerase chain reaction, LCR, etc. using thermostable polymerase, ligase, or Klenow fragment.). The reaction mixture comprises at least one deoxynuleotides and a deoxynuleotide that is labeled for detection, and it is required that the primer hybridizes to the target single stranded nucleic acid. Detection is typically performed using fluorescence, chemiluminescence, colorimetrically, electrically or combination thereof (thus, the deoxynucleotide is labeled with fluorophore, chemiluminophore, enzyme, radioactive or biotin, digoxin, or an electroactive label). Of course, it should be recognized that multiple fluorescent dyes can be used (*e.g.*, for determining a homozygote or a heterozygote, or for performing complex/multiplex analysis). Labeled or unlabeled deoxynuleotides can be used sequentially or simultaneously.

The following references (each of which is incorporated by reference herein) are provided to reflect the level of general knowledge in the art. Basic Planning for Quality (Westgard QC, Inc.: 7614 Gray Fox Trail, Madison WI 53717). Basic QC Practices, Second Edition (Westgard QC, Inc.: 7614 Gray Fox Trail, Madison WI 53717), and U.S. Pat. Nos. 5846710, 6498012, 6503718, 6013431, 6479242, 5726015, 6355433, 6511803, and 6503718.

Thus, specific embodiments and applications of improved biochip configurations and methods have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the present disclosure. Moreover, in interpreting the specification, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

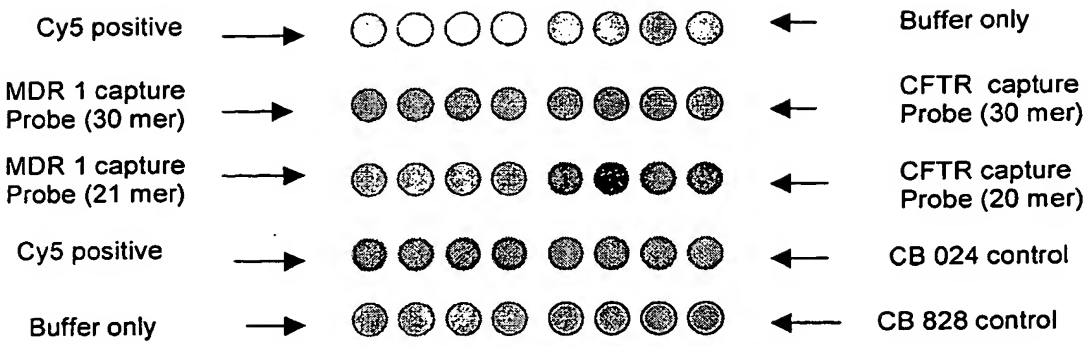

Figure 1

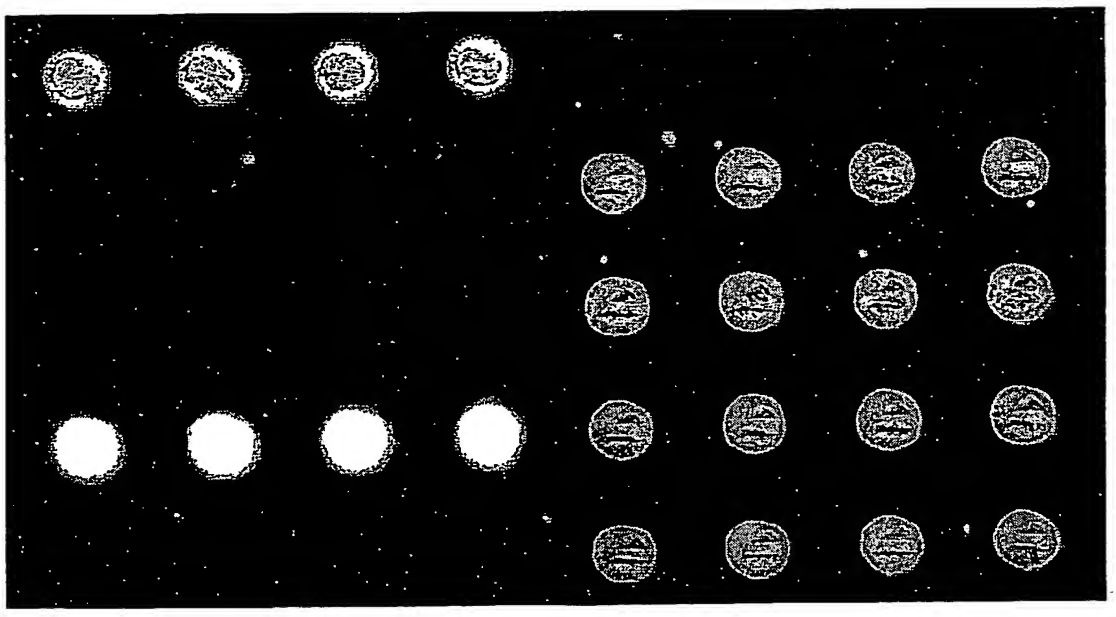

Figure 2

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